

A New Species of the Genus *Tylototriton* (Amphibia: Urodela: Salamandridae) from the Southern Dabie Mountains in Anhui Province

Lifu QIAN^{1**}, Xiaonan SUN^{1**}, Jiaqi LI², Weibo GUO², Tao PAN¹, Xing KANG¹, Hui WANG¹, Jianping JIANG³, Jun WU^{2*} and Baowei ZHANG^{1*}

¹ Anhui Key Laboratory of Eco-engineering and Bio-technique, School of Life Sciences, Anhui University, Hefei 230601, Anhui, China

² Nanjing Institute of Environmental Sciences under the Ministry of Environmental Protection, Nanjing 210042, Jiangsu, China

³ Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, Sichuan, China

Abstract A new species of the genus *Tylototriton* is described, from Yuexi county, Anhui province, in the south of the Dabie Mountains. It is based on morphological and molecular analysis. The new species is identified as belonging to the *Tylototriton asperrimus* group and shares a number of similarities with *T. wenxianensis*, *T. broadoridgus* and *T. dabienicus*. The diagnostic characteristics of the new species are as follows: the head length is greater than the width of the head; bony ridges on the head are prominent and necked-in; the distal digit ends, ventral digits, peripheral area of the cloaca and the tail's lower edge are orange. The result from the molecular analysis of the genus *Tylototriton* (including the type specimen of the new species) based on three mitochondrial genes (*ND1*, *ND2* and *CYTb*) indicated that the new species was close to *T. wenxianensis*, *T. dabienicus*, and *T. broadoridgus*, but formed an independent clade. This result was consistent with the morphological analysis above, which supports the theory that the population distributed in the south of the Dabie Mountains, namely in from Yuexi county, Anhui province, represented a distinct species, *Tylototriton anhuiensis* sp. nov.

Keywords new species, *Tylototriton anhuiensis* sp. nov., southern Dabie Mountains, taxonomy, morphology, molecular analyses

1. Introduction

The salamandrid (Asian newt) genus *Tylototriton*, established by Anderson in 1871, was widely distributed from the eastern Himalayas, Indochina, to central and southern China (Fei *et al.*, 2006; Stuart *et al.*, 2010; Fei *et al.*, 2012; Forst, 2016; Le *et al.*, 2015). Currently, 24 species are included in the genus *Tylototriton*. According

to previous studies, this genus was classified into two groups or subgenera: the *Tylototriton verrucosus* group and the *T. asperrimus* group (Fei *et al.*, 2005, 2006), corresponding to the two subgenera, *Tylototriton* and *Yaotriton* respectively (Dubois and Raffaelli, 2009). The *T. verrucosus* group is characterized by the presence of orange spots in the cranial region, on the body, tail, and dorsal ridge, or lateral dorsum (Yang *et al.*, 2014), and contains thirteen species (Anderson, 1871; Fang and Chang, 1932; Liu, 1950; Nussbaum *et al.*, 1995; Zhao *et al.*, 2012; Nishikawa *et al.*, 2014; Khatiwada *et al.*, 2015; Phimmachak *et al.*, 2015; Le *et al.*, 2015). The *T. asperrimus* group, is characterized by the lack of orange spots in the cranial region, body, tail, and dorsal ridge or lateral dorsum, and includes eleven species: *T.*

* Corresponding author: Prof. Baowei ZHANG, from the School of Life Sciences, Anhui University, China, with his research focusing on Taxonomy, evolution and phylogeography of amphibians; Prof. Jun WU, from Nanjing Institute of Environmental Sciences under the Ministry of Environmental Protection, with his research focusing on Nature conservation and biodiversity.

** These authors contributed equally to this paper.

E-mail: zhangbw@ahu.edu.cn (Baowei ZHANG); wujun@nies.org (Jun WU)

Received: 16 February 2017 Accepted: 27 March 2017

asperrimus, *T. notialis*, *T. hainanensis*, *T. vietnamensis*, *T. panhai*, *T. liuyangensis*, *T. lizhenchangi*, *T. broadoridgus*, *T. wenxianensis*, *T. dabienicus* and *T. ziegleri* (Fei *et al.*, 1984; Chen *et al.*, 2010; Shen *et al.*, 2012; Yang *et al.*, 2014; Nishikawa *et al.*, 2014). However, the membership of these two groups or subgenera is not stable, especially for the *T. asperrimus* group. In recent years, 8 species have been newly described (Bohme *et al.*, 2005; Stuart *et al.*, 2010; Chen *et al.*, 2010; Shen *et al.*, 2012; Hou *et al.*, 2012; Nishikawa *et al.*, 2013a; Nishikawa *et al.*, 2013b; Yang *et al.*, 2014), which indicate that some cryptic species exist within the subgenus *Yaotriton* (Fei *et al.*, 1984, 1990).

On the other hand, the status of some of the members of *Yaotriton* is still to be confirmed. In the early 1980s, some specimens of the genus *Tylototriton* were collected in the Wuling Mountains in Sangzhi County, Hunan, China, and they were identified as *T. asperrimus* (Shen, 1989) based on the presence of “^” shaped vomerine tooth rows, and a pair of obvious lateral bony ridges on the head which referred to existing documents (Hu *et al.*, 1973, 1977; Tian and Jiang, 1986; Shen *et al.*, 2012). Fei *et al.* (1984) collected specimens of *T. asperrimus* from Wenxian County in Gansu, Pingwu County in Sichuan and Mt. Leigong in Guizhou, which were identified as a new subspecies *T. asperrimus wenxianensis* based on the morphological characters being different from the topotype of *T. asperrimus*. Latterly, the new subspecies was upgraded to species rank, i.e., *T. wenxianensis* by Fei *et al.* (1990). In addition, the *T. asperrimus* species from Sangzhi County, Hunan province was also re-identified as *T. wenxianensis* (Fei *et al.*, 2005, 2006, 2010). Latterly, the Sangzhi population was described as a new species (*T. broadoridgus*) based on molecular and morphological analysis (Shen *et al.*, 2012). Chen *et al.* (2010) described a new subspecies, *T. wenxianensis dabienicus*, based on ten female specimens and 1 larva from the north Dabie Mountains in Henan province, China, which was classified as *T. wenxianensis* previously by Fei *et al.* (2005, 2006, 2010). Later, Shen *et al.*, 2012 proposed that this subspecies was likely to be reclassified as *T. dabienicus* based on molecular phylogenetic analysis of the genus *Tylototriton* (Shen *et al.*, 2012). Besides, the phylogenetic status of those specimens collected from the southern Dabie Mountains (SDM) also needs to be proved.

Hu (1989) collected *T. asperrimus* from the southern Dabie Mountains, Yuexi county, Anhui province, China, and reported a new discovery of *T. asperrimus* in Anhui province (Chen, 1991). Comparing this with the topotype

of *T. asperrimus*, Fei *et al.*, 2006 found the external morphological characteristics of those specimen from SDM were different from the former (*T. asperrimus*), but they were similar to *T. wenxianensis* (Fei *et al.*, 2006). Combined with the floristic characteristics of Amphibians in Anhui province and the geographical distribution of the *Tylototriton* species, they suggested the specimen from Yuexi county should be revised as *T. wenxianensis* (Fei *et al.*, 2006). Based on what Chen (1991) described, the head length is greater than the head width and the peripheral area of the cloaca is red-orange. So the morphology of the species from Yuexi county were similar to *T. dabienicus*, but different from *T. wenxianensis* (Chen *et al.*, 2010). However, the female total length and diameter of egg granules of *T. dabienicus* is significantly greater than the female specimen from Yuexi county (Chen *et al.*, 2010). Therefore, further morphology and molecular analysis needs to be done to confirm the taxa of the *Tylototriton* species from Yuexi county.

Recently, considering the research problems of previous studies, we reviewed the taxon status of the *Tylototriton* population from the southern Dabie Mountains. We systematically studied the specimens of *Tylototriton* from Yaoluoping National Nature Reserve in Yuexi county, Anhui Province of China, and recorded that distinct morphological and molecular genetic differences were to be found between the Yuexi population and the others, as well as among other known species of the *T. wenxianensis* group and *T. dabienicus*, supporting the theory that the Yuexi Population represented a previously unrecognized species.

2. Materials and Methods

2.1 Sampling The fieldwork was conducted from 2013 to 2016 in Yaoluoping National Nature Reserve in Yuexi county, Anhui Province of China (Figure 1). 32 specimens were also collected in the southern Dabie Mountains, Anhui province, China. Specimens were fixed in 99% ethanol, later transferred to 70% ethanol for permanent storage, and then deposited in the Anhui Key Laboratory of Eco-engineering and Bio-technique, School of Life Sciences, Anhui University, China.

2.2 Molecular analysis

2.2.1 Gene sequencing and collection A total of 32 DNA samples were extracted using a standard proteinase K/phenol-chloroform protocol (Sambrook *et al.*, 1989). The EasyPure PCR Purification Kit (TransGene) was used to purify each DNA extraction. We obtained sequence data of NADH dehydrogenase subunit 2 (*ND2*) and

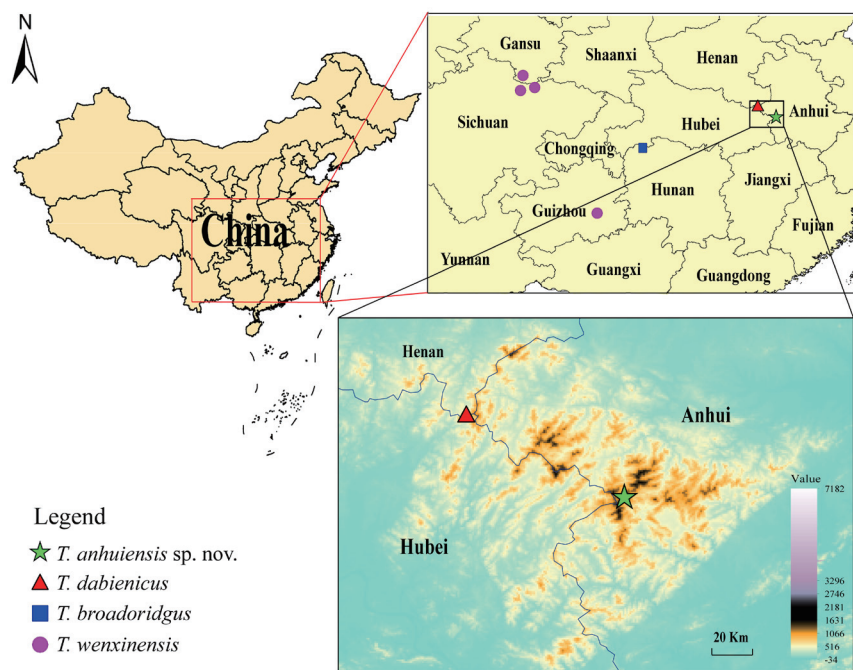


Figure 1 Localities for the *Tylototriton anhuiensis* sp. nov., and distribution for its three closely-related species. *T. wenxianensis* distributed in Gansu province (Wenxian county), Sichuan province (Ping wu and Qing chuan county) and Guizhou (Leishan county) following Fei *et al.* (2006). The *T. broadoridgus* distributed in Sangzhi county, following Yang *et al.* (2014). The *T. dabienicus* distributed in Shangcheng county, following Chen *et al.* (2010).

Table 1 Primers used in PCR amplification and sequencing in this study.

Primer	Primer Sequence	Size (bp)	Annealing (°C)	Source
ND2-4F	5'-TATGAGTACGAGCATCATACCC-3'	1407	53	This study
ND2-4R	5'-CTTCTGCTTAAGACTTTGAAGGTC-3'		54	This study
ND2-5F	5'-CAACATAACCTGACGAGTAGC-3'	1089	52	This study
ND2-5R	5'- GCGGTCGGTTAGAAGTATAGT-3'		52	This study
CYTB-13F	5'- CACACAATAACTACTAAACAGCTG-3'	1162	52	This study
CYTB-13R	5'- GGGTGAAGTTTCTGGGTCG-3'		54	This study
CYTB-14F	5'- AGGCTCAAAACAACCCAACAG-3'	756	52	This study
CYTB-14R	5'- AAGGGGTAGTTTAGTCCCAG-3'		52	This study

Cytochrome *b* (*CYTB*) of mitochondrial DNA (mtDNA) by four pairs of primers (Table 1), which were designed using Premier 5.0 (Clarke and Gorley, 2001) based on *T. wenxianensis* (EU880341), *T. asperrimus* (EU880340) and *T. verrucosus* (NC017871).

All PCRs were performed with the same conditions in 50μL: 1μL genomic DNA (concentration 20–80 ng/μL), 25μL 2×Easy*Taq* PCR SuperMix polymerase (TransGen Biotech, containing 1.25U Ex *Taq*, 0.4mM dNTP, 4mM Mg²⁺) and 1μL of each of the primers; pure molecular biology grade water was added to reach the appropriate volume. The amplification protocol included an initial denaturation step of 95°C for 5 min; this was followed by 33 cycles of denaturation at 95°C for 30s, primer annealing at 52–54°C for 40s, and an extension

at 72°C for 60–80s, with a final extension at 72°C for 10 min. PCR products were purified using an EasyPure PCR Purification Kit (TransGene), and sequenced using previous primers and the BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems) following the manufacturer’s instructions on an ABI Prism 3730 automated sequencer. New sequences were deposited in GenBank under accession numbers KY321388–KY321423 (Table S1).

In the present study, the haplotypes were identified using DnaSP v5.10.1 (Librado and Rozas, 2009). The *ND1*, *ND2* and *CYTB* gene sequences of congeneric species in *Tylototriton* were downloaded from GenBank (Table S1). In total, 119 sequences were used in the present study, and they involved 89 individuals of 24

species (Table S1).

2.2.2 Phylogenetic and divergence time analyses The phylogenetic trees of 4 haplotypes and 23 congeneric species in *Tylototriton* were reconstructed using Bayesian inference (BI) and Maximum likelihood (ML) methods, with *Pleurodeles waltl* and *Echinotriton andersoni* as outgroups (Zhang *et al.*, 2008). All DNA sequences obtained in this study were aligned automatically using Clustal X version 1.8 with default settings (Thompson *et al.*, 1997). Ahead of phylogenetic analysis, the software MrModeltest 2.3 (Nylander, 2004) was used to find the best-fit nucleotide substitution model of each gene using Akaike's Information Criterion (Akaike, 1974), and these models (GTR+G: *CYTb*; GTR + I + G: *ND1* and *ND2*) were selected, the optimal model being implemented in all downstream analysis. Bayesian phylogenetic analysis was performed on combined mitochondrial datasets (separated into different partitions with a mixed-model approach) using MrBayes 3.2.2 (Ronquist, and Huelsenbeck, 2003). Two parallel runs of Markov Chain Monte Carlo (MCMC) analyses for 1 million generations were conducted and tree sampling every 1,000 generations; in addition 10% of the initial samples were discarded as "burn-in". The ML method based on the partitioned data was performed in RaxML v7.2.6 (Stamatakis, 2006). The GTRCAT model was applied to the three partitions. Node support values were estimated by the rapid bootstrap with 1,000 replicates.

To estimate divergence times in *Tylototriton*, we applied a Bayesian MCMC method based on mitochondrial genomes (*ND2*), which employs a relaxed molecular clock approach, as implemented in BEAST 1.7.4 (Drummond *et al.*, 2012). Three nodes were constrained by extracting information from the fossil record to calibrate the phylogenetic tree (Zhang *et al.*, 2008): (1) the divergence time between *Pleurodeles* and (*Echinotriton* + *Tylototriton*) was about 45.1 Mya; (2) the divergence time between *Echinotriton* and *Tylototriton* was about 26.7 Mya; and (3) "*Yaotriton*" is suggested to have emerged around 14.2 Mya, corresponding to a 95% CI of 5.7–22.2 Mya. We assumed a relaxed uncorrelated log normal model of lineage variation, and a Yule Process prior to the branching rates, based on the HKY+I +G model, and as recommended by MrModeltest 1.0 b (Nylander, 2003). Four replicates were run for 100,000,000 generations with tree and parameter sampling occurring every 1,000 generations for the first 10% of samples that were discarded as burn-in. All parameters were assessed by visual inspection using Tracer v. 1.5 (Rambaut and Drummond, 2007). The tree

was generated and visualized with TreeAnnotator v. 1.6.1 (Rambaut and Drummond, 2010) and FigTree v. 1.3.1 (Rambaut, 2009), respectively.

2.2.3 Median-joining network and genetic distance A median-joining network was constructed to depict relationships among haplotypes using Network 4.6.1.2 (Bandelt *et al.*, 1999). Pairwise divergences (uncorrected *p*-distance) between *Tylototriton* species on *ND2* sequences were calculated using the Kimura 2-Parameter model in MEGA 5.0 (Tamura *et al.* 2011).

2.3 Morphological analyses Morphological measurements were made using a digital dial caliper to the nearest 0.01 mm. The following 14 measurements were taken for morphometric analyses (Fei *et al.*, 1999): total length (TOL, from tip of snout to tip of tail), snout-vent length (SVL, from tip of snout to posterior edge of vent), head length (HL, from jugular fold to snout tip), head width (HW, maximum head width), snout length (SL, from the tip of the snout to the anterior corner of the eye), trunk length (TRL, from the jugular plica to the anterior tip of the vent), interorbital space (IOS, least distance between upper eyelids), diameter of eye (ED, diameter of the exposed portion of the eyeball), tail length (TL, from posterior edge of the vent to tail tip), maximum tail height (TH, maximum height of the tail between the top and bottom margins), width of tail-head (TW, width of the base of the tail), length of fore leg (FLL, from axilla to anterior limb tip of the longest toe), length of hind leg (HLL, from groin to tip of the longest toe), and axilla-groin distance (AGD, distance between axilla and groin). Morphological differences between populations were examined by Student's *t*-test (for parametric data), based on SVL and 13 ratio values to SVL (R, %). The significance level was set at $P < 0.05$. The Principal Component Analysis (PCA) was used to look at the overall morphological variation by using SPSS Inc. version 15.0.

3. Results

3.1 Molecular analyses

3.1.1 Phylogenetic reconstructions and divergence time analyses Topologies were recovered from the Bayesian inference (BI) analyses of combined mtDNA (*ND1*, *ND2* and *CYTb*) for 24 *Tylototriton* species with highly posterior probability values of most nodes (Figure 2A). These 24 species were divided into two major lineages, lineage A and lineage B, which corresponded to subgenus *Tylototriton* and subgenus *Yaotriton*, respectively. Within subgenus *Yaotriton*, 12 species were

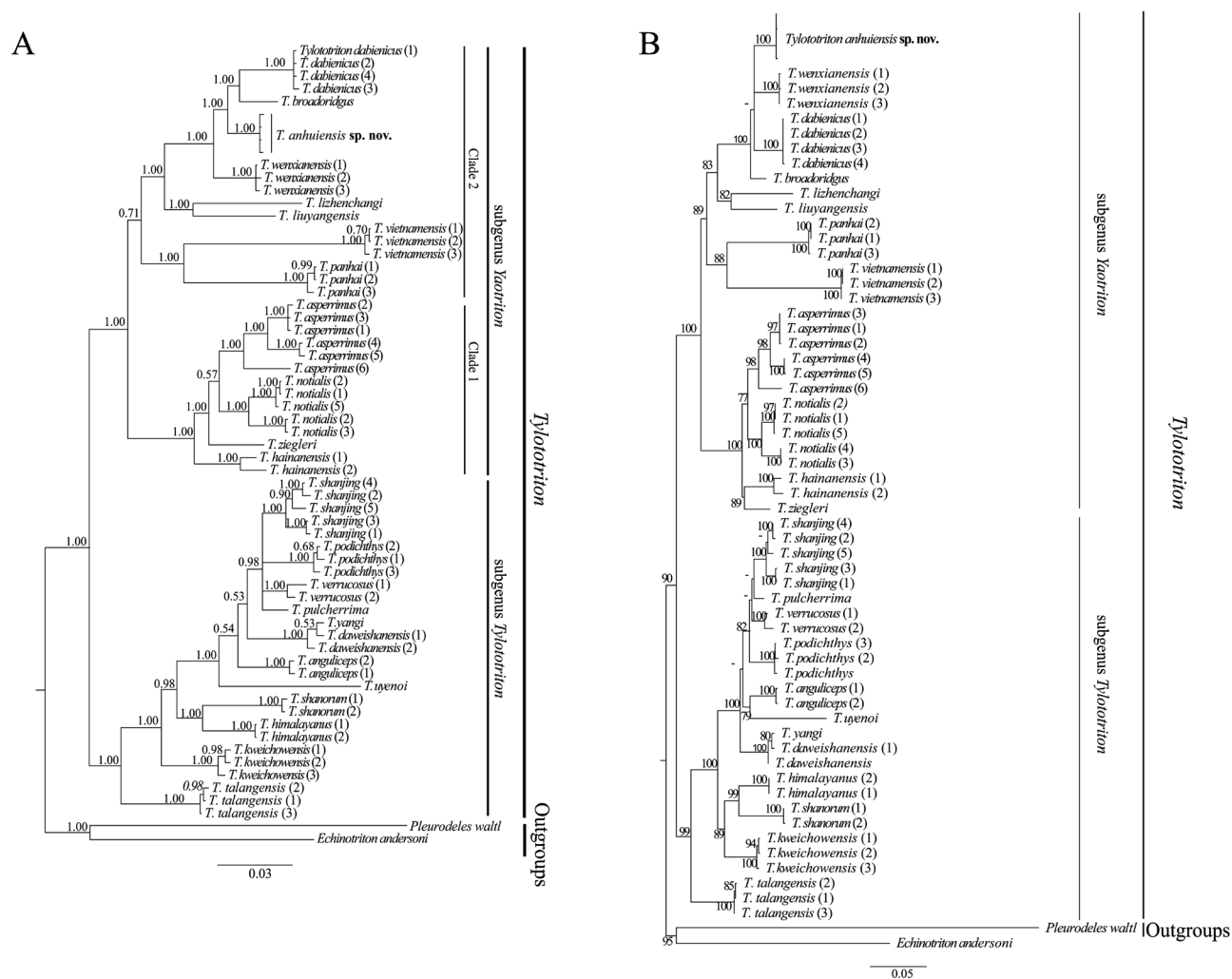


Figure 2 Bayesian inference (BI) tree based on combined mtDNA sequences (A) and the phylogenetic tree constructed by the maximum likelihood (ML) methods based on mtDNA ND2 (B). Values on branches of the trees are bootstrap support (bs) values from Bayesian posterior probabilities (bpp) and the Maximum parsimony analysis. * indicates the value < 75.

divided into two major clades: *T. hainanensis*, *T. zieglerei*, *T. notialis* and *T. asperimus* were clustered into clade 1 and the other 8 species were clustered into clade 2. In the inner clade 2 of lineage B, four haplotypes recognized from all Yuexi populations were mixed together and formed a monophyletic clade (BPP = 1.00) which were nested with *T. wenxianensis* and *T. broadoridgus* (BPP = 1.00). Four individuals of *T. dabienicus* were clustered with *T. broadoridgus* (BPP = 1.00). The phylogenetic tree from maximum likelihood (ML) methods also shows the same topologies (Figure 2B). Within subgenus *Yaotriton*, four haplotypes recognized from all Yuexi populations were mixed together. In addition, the species from *T. wenxianensis* and *T. broadoridgus* were also mixed together and formed an independent clade.

The molecular analyses of the ND2 gene also showed the estimated divergence times of the 24 species present

in the *Tylototriton* genus (Figure 3). The time elapsed since the most recent common ancestor (MRCA) of *Tylototriton* was estimated as 17.02 Ma (i.e., 95% CI, 11.84–23.21 Ma). The MRCA of subgenus *Yaotriton* was 13.62 Ma (i.e., 95% CI, 9.43–18.56 Ma), and the split between *T. anhuiensis* and both *T. broadoridgus* and *T. dabienicus* was estimated at 3.68 Ma (i.e., 95% CI, 2.10–5.80 Ma) (Figure 3).

3.1.2 Median-joining network and genetic distance

The median-joining network showed a clear pattern with obvious phylogenetic structure in accordance with the divergent time tree (Figure 4). Two groups were distinguished from the network analysis: group A was composed of *T. panhai*, *T. lizhenchangii* and *T. liuyangensis*, while group B contained *T. wenxianensis*, *T. dabienicus*, *T. broadoridgus* and four haplotypes. Within group A, *T. panhai* was more diverged from the other two

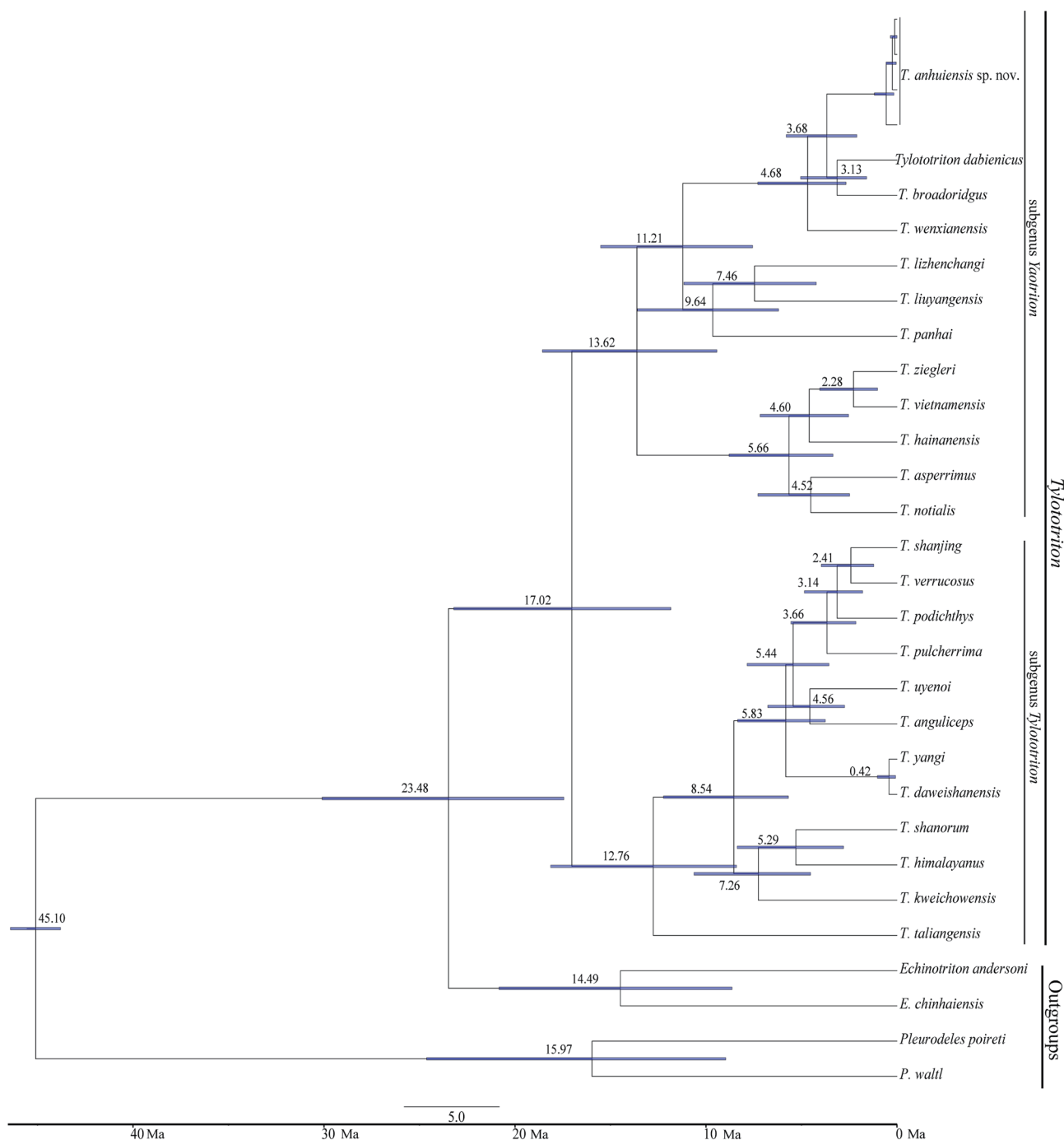


Figure 3 Time-calibrated tree of *Tylotriton* based on *ND2* sequences. The values indicate the split time was calculated by BEAST 1.7.4. The gray bars through these nodes indicated 95% highest posterior densities for estimates.

species. In group B, four haplotypes clustered together very closely to *T. dabienicus* and *T. broadoridgus*. There were at least 38 step mutations between the four haplotypes and *T. dabienicus*, but only 34 step mutations between *T. broadoridgus* and *T. dabienicus*. Within group B, *T. wenxianensis* was close to the four haplotypes and clustered into one clade, but there were at least 42 step mutations between them.

Interspecific genetic uncorrected *p*-distance of *ND2* in the genus *Tylotriton* species ranged from 0.4%–15.9% (Table 2). The *p*-distance between the Yuexi population and the other species ranged from 3.2% to 13.9%. The genetic distance between the Yuexi population and *T. dabienicus* (4%) is larger than *T. broadoridgus* to *T. dabienicus* (3.6%).

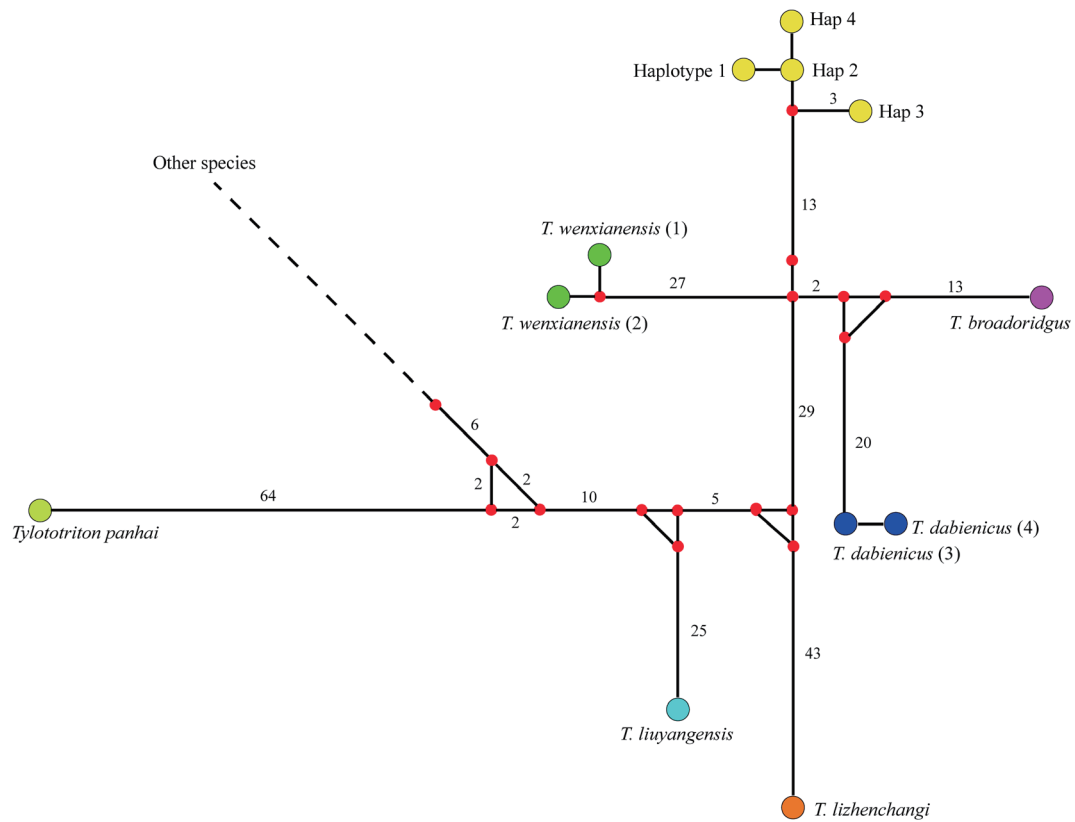


Figure 4 Median-joining network based on *ND2* sequences among haplotypes of *T. anhuiensis* sp. nov., and other congeneric species in *Tylototriton*. Length of branches is proportional to the number of changes from one species to the following, with a number next to the branch representing more than one mutation step.

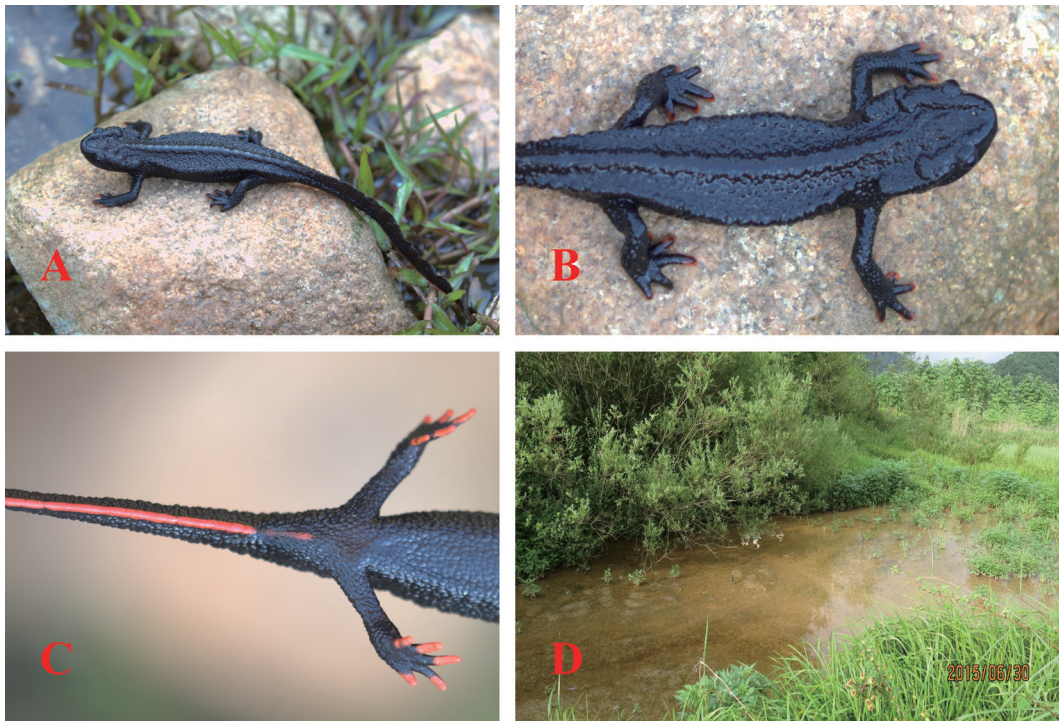


Figure 5 Photos of *T. anhuiensis* sp. nov., and the habitat. (A) Dorsal view of *T. anhuiensis* sp. nov.; (B) dorsal view of the head and trunk; (C) cloaca and ventral view of tail; (D) habitat of *T. anhuiensis* sp. nov.

Table 2 Genetic distances among the 24 species of the genus *Tylotriton*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1. <i>T. anguliceps</i>																							
2. <i>T. asperrimus</i>	0.124																						
3. <i>T. dawuoshanensis</i>	0.044	0.109																					
4. <i>T. verrucosus</i>	0.047	0.127	0.045																				
5. <i>T. taliangensis</i>	0.087	0.096	0.08	0.077																			
6. <i>T. podichthy</i>	0.054	0.118	0.053	0.042	0.078																		
7. <i>T. himalayanus</i>	0.073	0.126	0.073	0.069	0.075	0.072																	
8. <i>T. pulcherrima</i>	0.043	0.12	0.041	0.025	0.073	0.038	0.065																
9. <i>T. kweichowensis</i>	0.063	0.108	0.065	0.06	0.064	0.062	0.054	0.054															
10. <i>T. shanorum</i>	0.074	0.126	0.075	0.068	0.085	0.083	0.055	0.071	0.065														
11. <i>T. uyenoi</i>	0.079	0.147	0.083	0.081	0.106	0.093	0.095	0.073	0.088	0.103													
12. <i>T. shanying</i>	0.05	0.121	0.043	0.027	0.08	0.039	0.072	0.031	0.06	0.07	0.083												
13. <i>T. yangi</i>	0.045	0.11	0.004	0.045	0.082	0.054	0.073	0.041	0.065	0.075	0.085	0.043											
14. <i>T. notialis</i>	0.117	0.051	0.111	0.122	0.099	0.118	0.116	0.112	0.105	0.117	0.148	0.121	0.115										
15. <i>T. panha</i>	0.143	0.118	0.136	0.143	0.111	0.136	0.13	0.129	0.114	0.135	0.159	0.14	0.139	0.109									
16. <i>T. lizhenchangi</i>	0.134	0.115	0.12	0.126	0.101	0.12	0.122	0.115	0.113	0.128	0.141	0.121	0.122	0.107	0.1								
17. <i>T. ziegleri</i>	0.118	0.044	0.107	0.123	0.1	0.118	0.118	0.113	0.098	0.121	0.151	0.117	0.108	0.051	0.107	0.109							
18. <i>T. hainanensis</i>	0.111	0.052	0.1	0.112	0.089	0.108	0.105	0.1	0.09	0.112	0.143	0.108	0.101	0.051	0.101	0.099	0.044						
19. <i>T. vietnamensis</i>	0.118	0.046	0.11	0.125	0.106	0.119	0.122	0.113	0.1	0.123	0.152	0.117	0.108	0.05	0.104	0.111	0.023	0.042					
20. <i>T. liuyangensis</i>	0.109	0.098	0.103	0.111	0.094	0.106	0.108	0.103	0.098	0.11	0.139	0.112	0.103	0.094	0.1	0.076	0.092	0.087	0.089				
21. <i>T. wenxianensis</i>	0.116	0.094	0.103	0.105	0.091	0.104	0.113	0.096	0.102	0.12	0.138	0.105	0.105	0.096	0.11	0.086	0.097	0.095	0.103	0.077			
22. <i>T. broadridgus</i>	0.115	0.084	0.102	0.108	0.092	0.105	0.11	0.099	0.095	0.112	0.135	0.106	0.102	0.087	0.099	0.08	0.083	0.081	0.087	0.074	0.039		
23. <i>T. dabienicus</i>	0.132	0.1	0.118	0.119	0.098	0.117	0.119	0.112	0.106	0.12	0.146	0.114	0.118	0.1	0.101	0.094	0.097	0.096	0.103	0.08	0.052	0.036	
24. <i>T. anhuiensis</i>	0.114	0.087	0.106	0.113	0.091	0.105	0.113	0.104	0.1	0.114	0.139	0.107	0.103	0.088	0.099	0.086	0.086	0.085	0.092	0.076	0.043	0.032	0.04

3.2 Morphological comparisons The Yuexi Population has a series of morphology characters matching those of the subgenus *Yaotriton*, including vomerine teeth forming a “^” shape, a pair of prominent lateral bony ridges on the head, rough dorsal and ventral skin covered with warts, a completely black body, and the lack of an orange circular spot on the dorsal surface of the body (Figure 5A). The subgenus *Yaotriton* can be morphologically classified into two distinctly different groups (Yang *et al.*, 2014). One group contains *T. panhai*, *T. liuyangensis*, *T. wenxianensis*, *T. broadoridgus*, *T. dabienicus* and the Yuexi population. The salamanders in this group are characterized by the dense tubercles on the lateral trunk that form continuous nodule-like warts. The second *Yaotriton* group consists of *T. asperrimus*, *T. notialis*, *T. hainanensis*, *T. lizhenchangi*, *T. vietnamensis* and *T. ziegleri*. This group is characterized by the presence of relatively large tubercles among the nodule-like warts on the lateral dorsum, and a quite distinct relatively wide or relatively narrow gap between large warts. The main morphological differences between the Yuexi Population and the other three closely related species (*T. wenxianensis*, *T. broadoridgus* and *T. dabienicus*) is that the head length greater is than the width (Figure 5B).

The Yuexi population differs from *T. wenxianensis* by having the characteristic head length greater than width of head, the peripheral area of the cloaca is orange, and the tail height is larger than the width at the base (Figure 5B, C). The head length of *T. wenxianensis* is equal to the head width, and the tail height is less than the width at the base. Also, the peripheral area of the cloaca is blackish-brown and similar to the color of body (Fei, 1984). Bony ridges on the head of *T. wenxianensis* are notable and not necked-in, is another characteristic difference from the Yuexi population. Also, the Yuexi population differs from *T. broadoridgus* by the length of the dorsal ridge being smaller than the diameter of eye, and head length is greater than the head width (Figure 5B). The head length of *T. broadoridgus* is equal to head width, and the dorsal ridge is subequal to eye diameter (Shen *et al.*, 2012). The major difference between the Yuexi population and *T. dabienicus* is the total length of the female. The TOL of the southern Yuexi population (129.4 ± 18.2) is smaller than *T. dabienicus* (145.4 ± 8.2) (t -test = 2.267, $P < 0.05$) (Chen *et al.*, 2010). In addition, in the lateral of Yuexi population, fine transverse striae are present between every two tubercles.

The results of the PCAs exhibited the overall morphological difference among the Yuexi population and its closely-related species. The first three factors

explained 84.69% of the total variance. The first factor alone explained 57.23% of the variance, with large loading for the following 14 characters (the load factor > 0.1): SVL, TOL, HL, HW, SL, TRL, IOS, ED, TL, TH, TW, FLL, HLL and AGS. The second factor explained another 16.16% of the total variance, with the following 6 large loading characters: HL, HW, SL, ED, TH and TW. For the third factor, 11.31% variance was explained with large loading for the following 7 characters: TOL, HL, HW, SL, IOS, TL and TW. We constructed PCA plots of the first principal component (PC1) versus the second (PC2) for the group (Figure 6). The PCA graph showed that the Yuexi population was clustered together and clearly separated from the closely-related species.

3.3 New species description

Systematics The results from the morphological and molecular systematic comparisons above reciprocally support that the new form from the southern Dabie Mountains should be recognized as a new species. We name the Yuexi population of “*T. wenxianensis*” as *Tylototriton anhuiensis* sp. nov. in this paper.

***Tylototriton anhuiensis* sp. nov.** (Table 3, Figure 5)

Holotype AHU-13-EE-006, adult male (Figure 5), SVL 69.96 mm, was collected by Tao Pan from Yaoluoping National Nature Reserve (N 30°59'22.19", E 116°06'13.66", at an elevation of 1166 m) in Yuexi county, Anhui province on 01 June, 2013.

Paratype five adult males (AHU-13-EE-003~005, 008, 009), four adult females (AHU-13-EE-001, 002, 007 and AHU-15-EE-001) and two larvae (AHU-13-EE-010, 011) were collected by Tao Pan and Chencheng Wang from the same place as the holotype on June, 2013 and August, 2015. Sixteen adult males (AHU-16-EE-001~004, 006, 007, 009~012, 014~019 and) and four adult females (AHU-16-EE-005, 008, 013, 020) were collected by Chencheng Wang and Ke Fang from the same place as the holotype on April, 2016.

The holotype and paratypes were deposited in the Anhui Key Laboratory of Eco-engineering and Biotechnology, School of Life Sciences, Anhui University, China.

Diagnosis the new species has a series of morphological characteristics different from other members of the genus *Tylototriton*: 1) the head length is greater than the width of the head; 2) the bony ridges on the head are notable and necked-in; 3) the tail length is less than the snout-vent length; 4) the ventral tail fin fold extends to the cloacal posterior margin; 5) the distal digit ends, ventral digits, peripheral area of cloaca and the tail's lower margin are orange; 6) relative length of toes: $3 > 4 > 2 > 5 > 1$.

Table 3 Measurements of adult specimens of *T. anhuiensis* sp. nov.

Item (mm)	Holotype (♂)	%	Paratype (♂)		%	Paratype (♀)		%
			Means±SD	Range		Means±SD	Range	
SVL	69.96		69.2±3.9	(59.4-74.8)		68.1±12.2	(51.0-91.6)	
TOL	134.48	192.2	132.4±7.6	(118.9-145.7)	191.3	129.4±18.2	(103.8-165.4)	190
HL	16.41	24.4	15.6±0.9	(14.3-17.4)	22.5	15.6±1.5	(14.4-19.1)	22.9
HW	14.57	22.3	14.4±0.6	(13.2-15.4)	20.8	14.4±1.3	(13.2-17.2)	21.1
SL	3.11	4.4	3.5±0.4	(3.0-4.5)	5.1	4.0±0.5	(3.4-4.9)	5.9
TRL	55.32	79.1	55.0±4.0	(45.0-60.0)	79.5	52.9±9.4	(37.8-68.3)	81.3
IOS	7.88	11.3	7.5±0.7	(6.3-8.7)	10.8	7.5±1.0	(6.1-9.1)	11
ED	3.19	4.6	3.7±0.3	(3.2-4.3)	5.3	3.4±0.4	(2.4-3.8)	5
TL	64.72	92.5	63.3±5.1	(53.2-72.3)	91.5	61.3±6.8	(52.9-73.8)	90
TH	6.04	8.6	7.3±0.7	(5.8-8.2)	10.5	6.7±0.9	(5.2-8.0)	9.8
TW	4.41	6.3	4.2±0.4	(3.3-4.9)	6.1	4.2±0.8	(3.6-6.1)	6.2
FLL	20.27	29	20.9±1.5	(18.5-23.6)	30.2	20.6±1.6	(18.8-24.0)	30.2
HLL	23.36	33.4	21.6±1.9	(17.0-23.7)	31.2	22.5±1.1	(21.0-24.6)	33
AGS	34.24	48.9	38.0±4.6	(26.7-43.1)	54.9	36.1±7.9	(27.8-51.4)	53

% is the ratio of each measurement to the SVL.

Description of the holotype (AHU-13-EE-006) an adult male, total length 134.68 mm, snout-vent length 69.96 mm (other measurements are given in Table 3). The head is compressed, longer than its width, and broader than the body width, (HL 16.41mm, HW 14.57 mm). The head slightly is concave on the top. The snout is nearly square, clearly extending beyond the lower lip. The external naris is slightly nearly snout. The bony ridges on the head are notable, extending from the dorsal region of the rostral side, and through the interior side of the upper eyelid to the occiput. The two bony ridges on the dorsal head surface form a “V” shape, which is low and flat, and connected with the dorsal ridge of body. The eyes protrude from the dorsolateral portion of the head and have oval eyelids. The oral fissure is flat, straight, and extends a distance greater than half of the length of the head. Fine teeth are presenting on the edge of the jaw, vomerine teeth forming a “ \wedge ” shape being positioned in the internal nares and extending posteriorly behind the orbit. The tongue is nearly round and entirely fixed at the base, but free at both lateral edges. The neck is rounded and thick, with a distinct neck groove.

The four limbs are relatively slender, and the hind limbs are lightly longer than the forelimbs. The distal tips of the limbs overlap when the fore and hind limbs are pressed along the trunk. The fingers and digits are compressed with none webbed. The relative length of 4 fingers is: $3>2>4>1$, and the relative length of 5 toes: $3>4>2>5>1$.

The tail length is less than the snout-vent length. The tail is notably compressed laterally, with relatively strong tail muscles. The dorsal fin fold of the tail, starting from

the tail base, is distinctly thin and high; while the ventral fin fold of the tail, starting from posterior of the cloaca, is thick and short. The tail height is greater than the width at the tail base, and the distal tail tip is obtuse and sharp. The cloaca is long and narrow, and the cloacal region is slightly apophysis.

The skin is extremely rough, and the body is covered with tubercles and warts. Only the labial margin, distal limbs, ventral limbs and the ventral edge of the tail are smooth. The dorsal ridge, running along the middle of the dorsum and extending from the neck to the base of the tail, is rough and relatively narrow, smaller than the diameter of the eye, with the anterior width being subequal to the posterior width. Each lateral corner of the dorsal ridge consists of a row of rough nodular tubercles. On the lateral dorsum of the body, tubercles and warts are big and closely arranged, appearing to form lines that extend from the shoulder to the base of the tail. Unlike the dorsal tubercles, the ventral tubercles and warts are relatively flat. The dorsal edge of the tail is covered with prominent tubercles, and the lateral tail and peripheral area of the cloaca contain warts.

Colour the entire specimen in life is almost black or blackish-brown, while the ventral color is a little lighter. Only the distal digit ends, ventral digits, the peripheral area of cloaca and the ventral edge of tail are orange (Figure 5C). The orange region between the ventral edge of the tail and the peripheral area of the cloaca is connected. The soaking specimen is black and the ventral color is blackish-brown. The orange coloration of the distal digit ends, ventral digits, the peripheral area of the cloaca and the ventral edge of the tail fades to lacte

(Figure 5C).

Secondary sexual characteristics the female cloacal hole is short and its inner cloacal wall has no papilla (Figure 7). The male has papilla on its inner cloacal wall and its cloacal orifice long slit (Figure 7). In the breeding season, the peripheral area of the cloaca is an obvious orange in the male, but the female cloacal region has fine orange dots that are more subtle than obvious. Also,

the cloacal region is slightly bulbous, and this feature becomes more evident during the breeding season in both male and female.

Variation all type specimens were collected from the type locality during the breeding season. The morphological characteristics of individuals are different because of the sexuality, size, and growth factors, which have been recorded detail in Table 2.

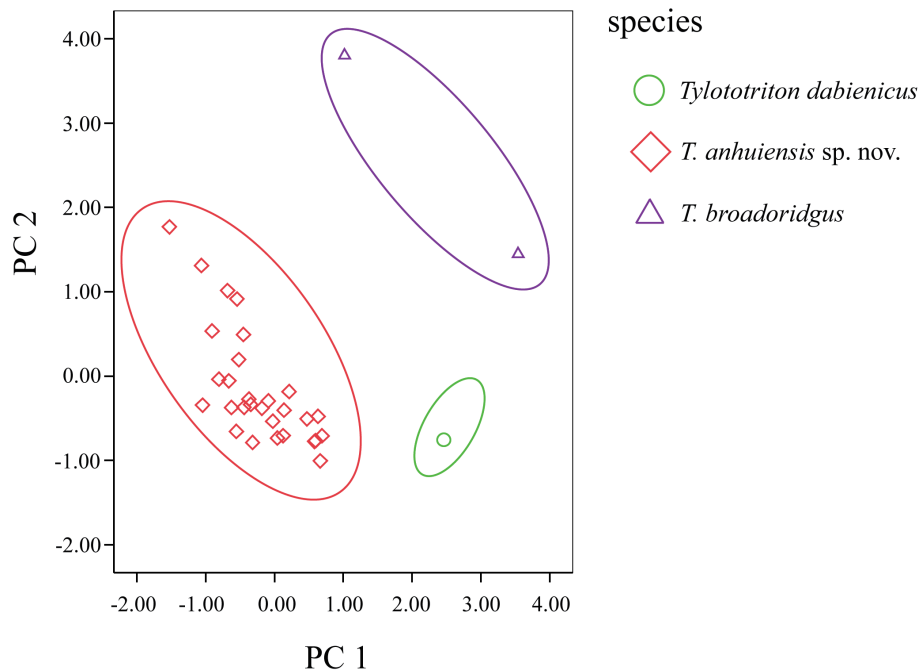


Figure 6 PCA plots of the first principal component (PC 1) versus the second (PC 2) for the *T. anhuiensis* sp. nov., and its closely-related species.

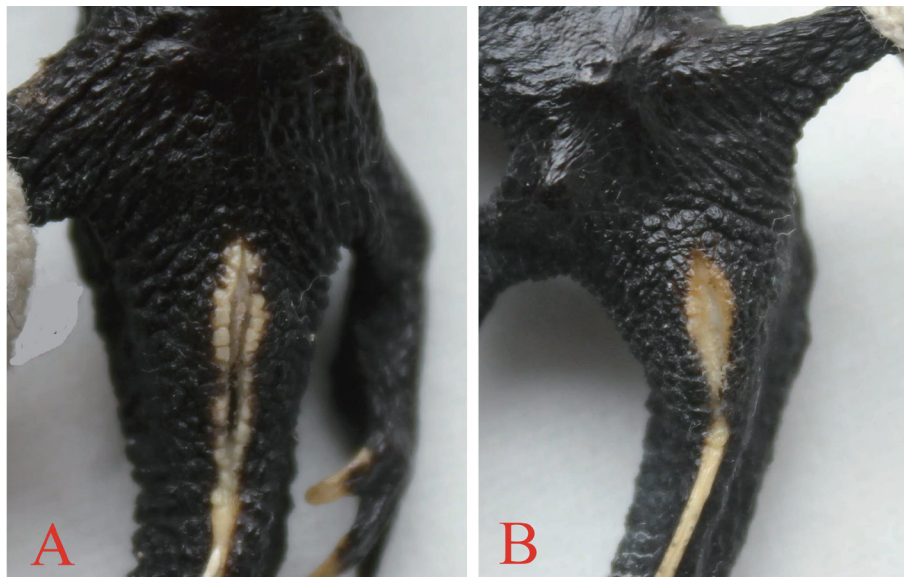


Figure 7 The cloaca of the male (A) and the female (B) of *T. anhuiensis* sp. nov.

Distribution and ecological habits the new species lives in a mountainous area at an elevation between 1000m to 1200m, in bamboo groves or where the land is covered with thick, dry branches and fallen leaves. The habitats are characterized as subtropical mountain forests. The adult salamanders are more land based in non-breeding seasons. But in the breeding season they can be found living in or near ponds, or wet soils between stones, rotting wet corn stalk heaps, and the soil of rice paddies (Figure 5D). They are rarely active during the day, and come out to feed in the evening, being most attracted to the humus at the bottom of ponds or damp places where the soil is rich. The species is very active before a storm, feeding on worms and flies and their larvae; they also eat spiders and other insects.

Larva larvae were collected from Yaoluoping National Nature Reserve in August 2013, and were deposited in the Anhui Key Laboratory of Eco-engineering and Biotechnology, School of Life Sciences, Anhui University, China.

The larval body is slender and 44.8mm in total length. The forelimbs and hindlimbs are clearly fully grown up, but the limbs appear to be weak, with locomotion primarily occurring through tail movement. The head is wide and flat with relatively large eyes and three pairs of external gills having obtuse anterior ends, while the body and tail are laterally compressed. The skin is smooth and the ventral side of the tail is almost white with a few scattered blackish-brown spots on the dorsal side of the tail. Costal grooves for muscles of larvae can be easily seen. The dorsal fin fold is comparatively tall and rises from the region between the posterior head and anterior body. The short and thick ventral fin fold of the tail extends from the cloaca to the end of the tail.

Etymology the name of the new species is derived from its current distribution range in the southern Dabie Mountains in Anhui province. The suggested English name is the Anhui Knobby Newt.

4. Discussions

Amphibians, especially salamanders, rarely wander away from their preferred environment and are highly associated with specific habitat requirements for breeding and larval growth (Zamudio and Wieczorek, 2007). Geographic isolation may cause gene flow to be interrupted, and the increasing genetic differentiation among the population can result in the formation of a new species (Eckert *et al.*, 2008; Chen *et al.*, 2010). The Dabie Mountains are lower in the south and higher in the north

and present a variety geomorphic features, located in the overlapping areas of the Palaearctic and Oriental, and its fauna change markedly with the change in elevation (Pan *et al.*, 2014). Because of this particularity of the natural environment, the diversity of species is plentiful and many cryptic species exist in the Dabie Mountains (Jiang *et al.*, 2006; Fei *et al.*, 2010; Huang *et al.*, 2012; Pan *et al.*, 2014; Pan *et al.*, 2015).

In our study, the results revealed that the Yuexi Population, which was previously known as a population of *T. asperrimus* (Chen, 1991) and latterly classified as *T. wenxianensis* (Fei *et al.*, 2006), is described as a distinct species based on three main factors: (1) both morphologically and genetically, the Yuexi population is very distinct from the closely-related species *T. wenxianensis*, *T. broadoridgus* and *T. dabienicus* (Tables 2–3; Figures 2, 3, 4, 6); (2) the split between Yuexi population and both *T. broadoridgus* and *T. dabienicus* occurred in the middle Pliocene (about 3.6 Ma) (Figure 3); (3) the geographic distribution of the Yuexi population and its three closely-related species does not overlap (Figure 1). Based on these analyses mentioned above, we conclude that the specimens collected from the southern Dabie Mountains belong to the subgenus *Yaotriton*, and that they are distinctly different from any of the known species in the genus *Tylotriton*. Thus, we suggest that this specimen from the southern Dabie Mountains should be recognized as a valid new species.

Acknowledgments We would like to thank for supporting the surveys the Administration Office of the Yaoluoping National Nature Reserve of Anhui. We thank Wenliang ZHOU, Zhonglou SUN, Ke FANG and Chencheng WANG for help in collecting specimens. We are also grateful for the support and assistance in molecular and morphological systematics analyses from Yanan ZHANG. This work was partly supported by the Graduate Student Academic Innovation Research Project of Anhui University (yqh100101) and the National Key Research and Development Programme (2016YFC1200700).

References

- Akaike H. 1974. A new look at the statistical model identification. IEEE transactions on automatic control, 19(6): 716–723
- Anderson J. 1871. Description of a new genus of newts from western Yunnan. In Proceedings of the zoological Society of London, Vol. 1871. 423–425
- Bandelt H. J., Forster P., Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol, 16: 37–48

- Bohme W., Schottler T., Nguyen T. Q., Kohler J.** 2005. A new species of salamander, genus *Tylototriton* (Urodela: Salamandridae), from northern Vietnam. *Salamandra-Bonn*, 41(4): 215–220
- Chen B. H.** 1991. The amphibian and reptilian fauna of Anhui. Hefei: Anhui science and technology press, 39–41 (In Chinese)
- Chen X. H., Wang X. W., Tao J.** 2010. A new subspecies of genus *Tylototriton* from China (Caudata, Salamandridae). *Acta Zootaxon Sinica*, 35: 666–670 (In Chinese)
- Clarke K. R., Gorley R. N.** 2001. Primer v5. Primer-E Ltd
- Drummond A. J., Suchard M. A., Xie D., Rambaut A.** 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol*, 29: 1969–1973
- Dubois A., Raffaëlli J.** 2009. A new ergotaxonomy of the family Salamandridae Goldfuss, 1820 (Amphibia, Urodela). *Alytes*, 26(1–4): 1–85
- Eckert C., Samis K., Lougheed S.** 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Mol Ecol*, 17(5): 1170–1188
- Fang P. W., Chang M. L. Y.** 1932. Notes on *Tylototriton kweichouensis* Unterstein with synopsis to species. *Sinensia*, 2(9): 111–122
- Fei L.** 1999. Atlas of amphibians of China. Zhengzhou: Henan science and technology press, 6–7 (In Chinese)
- Fei L., Hu S. Q., Ye C. Y., Huang Y. Z.** 2006. Fauna Sinica. Amphibia, Vol. I. Beijing: Science Press, 1–471 (In Chinese)
- Fei L., Ye C. Y., Jiang J. P.** 2012. Colored Atlas of Chinese Amphibians and Their Distributions. Chengdu: Sichuan Publishing House of Science and Technology, 78–94 (In Chinese)
- Fei L., Ye C. Y., Huan Y. Z., Jiang J. P., Xie F.** 2005. An illustrated key to Chinese amphibians. Chengdu: Sichuan Publishing House of Science and Technology, 340 (In Chinese)
- Fei L., Ye C. Y., Yang R. S.** 1984. A new species and new subspecies of the genus *Tylototriton* (Caudata: Salamandridae). *Acta Zool Sin*, 30(11), 85–91 (In Chinese)
- Fei L., Ye C. Y., Huang Y. Z.** 1990. The Key to Chinese Amphibian. Chongqing: Chongqing Branch of Science and Technology Literature Publishing House, 1–364 (In Chinese)
- Fei L., Ye C. Y., Jiang J. P.** 2010. Colored Atlas of Chinese Amphibians. Chengdu: Sichuan Publishing House of Science and Technology, 1–519 (In Chinese)
- Frost D.** 2016. Amphibian species of the world: an online reference. Version 6.0. American Museum of Natural History, New York, USA. Available at: <http://research.amnh.org/herpetology/amphibia/index.html>. (Assesses on Oct 11, 2016)
- Hou M., Li P. P., Lv S. Q.** 2012. Morphological research development of genus *Tylototriton* and primary confirmation of the status of four cryptic populations. *J Huangshan Univ*, 14: 61–65 (In Chinese)
- Hu S. Q., Zhao E. M., Liu C. C.** 1973. A survey of amphibians and reptiles Kweichow (= Guizhou) Province, including a herpetofaunal analysis. *Acta Zool Sin*, 19(2): 149–178 (In Chinese)
- Hu S. Q., Ye C. Y., Fei L.** 1977. Systematic Key to Amphibians of China. Beijing: Science Press, 1–93 (In Chinese)
- Huang X., Pan T., Han D. M., Zhang L., Hou Y. X., Yu L., Zheng H. M., Zhang B. W.** 2012. A new species of the genus *Protobothrops* (Squamata: Viperidae: Crotalinae) from the Dabie mountains, Anhui, China. *Asian Herpetol Res*, 3(3): 213–218
- Jiang J. P., Chen X. H., Wang B.** 2006. A new genus of family Ranidae from China - *Yerana* (Ranidae: Dicroglossinae). *Journal of Anhui Normal University (Natural Science)*, 29(5): 467–469 (In Chinese)
- Khatiwada J. R., Wang B., Ghimire S., Vasudevan K., Paudel S., Jiang J.** 2015. A New Species of the Genus *Tylototriton* (Amphibia: Urodela: Salamandridae) from Eastern Himalaya. *Asian Herpetol Res*, 6(4): 245–256
- Le D. T., Nguyen T. T., Nishikawa K., Nguyen S. L. H., Pham A. V., Matsui M., Bernardes M., Nguyen T. Q.** 2015. A new species of *Tylototriton* Anderson, 1871 (Amphibia: Salamandridae) from northern Indochina. *Curr Herpetol*, 34(1): 38–50
- Librado P., Rozas J.** 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25: 1451–1452
- Liu C. C.** 1950. Amphibians of Western China, V02. *Fieldiana: Zool Men*, 102–110 (In Chinese)
- Nishikawa K., Khonsue W., Pomchote P., Matsui M.** 2013a. Two new species of *Tylototriton* from Thailand (Amphibia: Urodela: Salamandridae). *Zootaxa*, 3737(3): 261–279
- Nishikawa K., Matsui M., Nguyen T. T.** 2013b. A new species of *Tylototriton* from northern Vietnam (Amphibia: Urodela: Salamandridae). *Curr Herpetol*, 32: 34–49
- Nishikawa K., Matsui M., Rao D. Q.** 2014. A new species of *Tylototriton* (Amphibia: Urodela: Salamandridae) from central Myanmar. *Nat Hist Bull Siam Soc*, 60(1): 9–22
- Nussbaum R. A., Brodie J. E. D., Datong Y.** 1995. A taxonomic review of *Tylototriton verrucosus* Anderson (Amphibia: Caudata: Salamandridae). *Herpetologica*, 257–268
- Nylander J.** 2003. MrModeltest 1.0 b. a simplified version of David Posada's "Modeltest 3.06". Uppsala: Department of Systematic Zoology
- Nylander, J.** 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University
- Pan T., Wang H., Hu C. C., Sun Z. L., Zhu X. X., Meng T., Meng X. X., Zhang B. W.** 2015. Species delimitation in the genus *Moschus* (Ruminantia: Moschidae) and its high-plateau origin. *PLoS one*, 10(8): e0134183
- Pan T., Zhou W. L., Shi W. B., Zhao K., Chen J. Y., Wang W. G., Chu J., Pu F. G., Gu C. M., Zhang B. W.** 2014. Species richness of amphibians and reptiles in Dabie Mountains, China. *Chinese Journal of Zoology*, 49(2): 195–206 (In Chinese)
- Phimmachak S., Aowphol A., Stuart B. L.** 2015. Morphological and molecular variation in *Tylototriton* (Caudata: Salamandridae) in Laos, with description of a new species. *Zootaxa*, 4006(2): 285–310
- Rambaut A.** 2009. FigTree version 1.3. 1. Computer program distributed by the author, website: <http://treebioedacuk/software/figtree/> (accessed January 4, 2011)
- Rambaut A., Drummond A.** 2007. Tracer. Version 1.5
- Rambaut A., Drummond A.** 2010. TreeAnnotator version 1.6. 1
- Ronquist F., Huelsenbeck J. P.** 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19(12): 1572–1574
- Sambrook J., Fritsch E. F., Maniatis T.** 1989. Molecular cloning.

- New York: Cold spring harbor laboratory press
- Shen Y. H.** 1989. A survey of the tailed amphibians in Hunan Province. *Chin wildlife*, (6): 77–84 (In Chinese)
- Shen Y. H., Jiang J. P., Mo X. Y.** 2012. A new species of the genus *Tylototriton* (Amphibia, Salamandridae) from Hunan, China. *Asian Herpetol Res*, 1(3): 21–30
- Stamatakis A.** 2006. RAxML-VI-HP: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22(21): 2688–2690
- Stuart B., Phimmachak S., Niane S., Robichaud W. G.** 2010. A new species in the *Tylototriton asperrimus* group (Caudata: Salamandridae) from central Laos. *Zootaxa*, 2650: 19–32
- Tamura K., Peterson D., Stecher G., Nei M., Kumar S.** 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*, 28: 2731–2739
- Thompson J. D., Gibson T. J., Plewniak F., Jeanmougin F., Higgins D. G.** 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res*, 25: 4876–4882
- Tian W. S., Jiang Y. M.** 1986. Identification manual of Chinese amphibians and reptiles. Beijing: Science Press, 1–164 (In Chinese)
- Yang D. D., Jiang J. P., Shen Y. H., Fei D. B.** 2014. A new species of the genus *Tylototriton* (Urodela: Salamandridae) from northeastern Hunan Province, China. *Asian Herpetol Res*, 5(1): 1–11
- Zamudio K. R., Wiczorek A. M.** 2007. Fine-scale spatial genetic structure and dispersal among spotted salamander (*Ambystoma maculatum*) breeding populations. *Mol Ecol*, 16(2): 257–274
- Zhang, P., Papenfuss, T. J., Wake, M. H., Qu, L., Wake, D. B.** 2008. Phylogeny and biogeography of the family Salamandridae (Amphibia: Caudata) inferred from complete mitochondrial genomes. *Mol Phylogenet Evol*, 49(2): 586–597
- Zhao T. Y., Rao D. Q., Liu N., Li B., Yuan S. Q.** 2012. Molecular phylogeny analysis of *Tylototriton verrucosus* group and description of new Species. *China Forest Sci*, 41: 85–89 (In Chinese)

Appendix

Table S1 Samples used in the molecular in this study.

ID	Species/ (No.) *	Voucher	Locality	GenBank accession No.			Sequence Source
				NDI	ND2	CYTb	
1.	<i>T. verrucosus</i> (1)	CAS 245290	-	KT304278	KT304278	-	Unpublished
2.	<i>T. verrucosus</i> (2)	CAS 245418	-	KT304279	KT304279	-	Unpublished
3.	<i>T. shanorum</i> (1)	CAS 230933	Taunggyi, Shan, Myanmar	-	AB922822	-	Nishikawa <i>et al.</i> 2014
4.	<i>T. shanorum</i> (2)	CAS 230940	Taunggyi, Shan, Myanmar	-	AB922823	-	Nishikawa <i>et al.</i> 2014
5.	<i>T. taliangensis</i> (1)	CAS 195126	Liangshan, Sichuan, China	DQ517853	DQ517853	-	Weisrock <i>et al.</i> 2006
6.	<i>T. taliangensis</i> (2)	-	-	-	-	EF627455	Unpublished
7.	<i>T. taliangensis</i> (3)	KIZ 05149	-	KT304302	KT304302	-	Phimmachak <i>et al.</i> 2015
8.	<i>T. kweichowensis</i> (1)	GZNU2007051802	-	-	FJ415597	-	Unpublished
9.	<i>T. kweichowensis</i> (2)	GZNU07060101	-	-	FJ415598	-	Unpublished
10.	<i>T. kweichowensis</i> (3)	MVZ2230371	-	-	DQ517851	-	Weisrock <i>et al.</i> 2006
11.	<i>T. shanjing</i> (1)	MVZ219763	Daquan -, Yunnan, China	DQ517852	DQ517852	-	Weisrock <i>et al.</i> 2006
12.	<i>T. shanjing</i> (2)	NMNS 3682	Jingdong, Yunnan, China	-	AB830721	-	Nishikawa <i>et al.</i> 2013b
13.	<i>T. shanjing</i> (3)	YN 0705201	Jingdong, Yunnan, China	KT304277	KT304277	-	Phimmachak <i>et al.</i> 2015
14.	<i>T. shanjing</i> (4)	CAS HERP:215071	Nu Jiang Pref, Yunnan, China	HM462054	HM462054	-	Stuart <i>et al.</i> 2010
15.	<i>T. shanjing</i> (5)	CIB-YN2009060638	Long chuan -, Yunnan, China	-	KT765172	KT765132	Khatiwada <i>et al.</i> 2015
16.	<i>T. himalayanus</i> (1)	CIB 201406287	Bagh Khor, Illam, Mechi, Nepal	-	KT765210	KT765170	Khatiwada <i>et al.</i> 2015
17.	<i>T. himalayanus</i> (2)	CIB 201406288	Bagh Khor, Illam, Mechi, Nepal	-	KT765211	KT765171	Khatiwada <i>et al.</i> 2015
18.	<i>T. anguliceps</i> (1)	NUOL 00420	-	KT304301	KT304301	-	Phimmachak <i>et al.</i> 2015
19.	<i>T. anguliceps</i> (2)	NCSM 82952	-	KT304300	KT304300	-	Phimmachak <i>et al.</i> 2015
20.	<i>T. podichthys</i> (1)	NCSM 77725	-	KT304295	KT304295	-	Phimmachak <i>et al.</i> 2015
21.	<i>T. podichthys</i> (2)	NCSM 77726	-	KT304294	KT304294	-	Phimmachak <i>et al.</i> 2015
22.	<i>T. podichthys</i> (3)	NCSM 77722	-	KT304296	KT304296	-	Phimmachak <i>et al.</i> 2015
23.	<i>T. yangi</i>	NMNS:3114	Pingbian, Yunnan, China	-	LC017831	-	Nishikawa <i>et al.</i> 2015
24.	<i>T. daweihsanensis</i> (1)	RDQ 201203002	Pingbian, Yunnan, China	-	LC017830	-	Nishikawa <i>et al.</i> 2015
25.	<i>T. daweihsanensis</i> (2)	RDQ 201203001	Pingbian, Yunnan, China	-	LC017829	-	Nishikawa <i>et al.</i> 2015
26.	<i>T. pulcherrima</i>	KUHE:46406	Yunnan, China	-	AB830738	-	Nishikawa <i>et al.</i> 2013b
27.	<i>T. uyenoi</i>	KUHE:19147	Doi Suthep, Chiang Mai, Thailand	-	AB830733	-	Nishikawa <i>et al.</i> 2013b
28.	<i>T. asperimus</i> (1)	CIB 70063	Longsheng, Guangxi, China	-	KC147816	-	Nishikawa <i>et al.</i> 2013a
29.	<i>T. asperimus</i> (2)	CIB200807055	Jinxiu, Guangxi, China	-	KC147815	-	Nishikawa <i>et al.</i> 2013a
30.	<i>T. asperimus</i> (3)	YPX 9918	-	KT304303	KT304303	-	Phimmachak <i>et al.</i> 2015
31.	<i>T. asperimus</i> (4)	2010052501	-	-	JF825873	-	Unpublished
32.	<i>T. asperimus</i> (5)	2010052502	-	-	JF825874	-	Unpublished
33.	<i>T. asperimus</i> (6)	VNMTAO1214	Thuong Tien, Hoa Binh, Vietnam	-	AB769531	-	Nishikawa <i>et al.</i> 2013a
34.	<i>T. hainanensis</i> (1)	CIB20081049	Dioluoshan, Hainan, China	-	KC147818	-	Nishikawa <i>et al.</i> 2013a
35.	<i>T. hainanensis</i> (2)	MVZ236632	Jianfengling, Hainan, China	DQ517850	DQ517850	-	Weisrock <i>et al.</i> 2006
36.	<i>T. notialis</i> (1)	FMNH:HERP:271121	Boualapha Dist, Khammouan, China	HM462062	HM462062	-	Stuart <i>et al.</i> 2010
37.	<i>T. notialis</i> (2)	FMNH:HERP:271120	Boualapha Dist, Khammouan, China	HM462061	HM462061	-	Stuart <i>et al.</i> 2010
38.	<i>T. notialis</i> (3)	NCSM 80315	-	KT304304	KT304304	-	Phimmachak <i>et al.</i> 2015

ID	Species/ (No.) *	Voucher	Locality	GenBank accession No.			Sequence Source
				ND1	ND2	CYTB	
39.	<i>T. notialis</i> (4)	VNMN:TAO1235	Phu Hoat, Nghe An, Vietnam	-	AB769536	-	Nishikawa <i>et al.</i> 2013a
40.	<i>T. notialis</i> (5)	FMNH:HERP:271122	Boualapha Dist, Khammouan, China	HM462063	HM462063	-	Stuart <i>et al.</i> 2010
41.	<i>T. ziegleri</i>	VNMN:3390	Quan Ba, Ha Giang, Vietnam	-	AB769539	-	Nishikawa <i>et al.</i> 2013a
42.	<i>T. panhai</i> (1)	NUOL 00421	-	KT304310	KT304310	-	Phimmachak <i>et al.</i> 2015
43.	<i>T. panhai</i> (2)	NUOL 00425	-	KT304311	KT304311	-	Phimmachak <i>et al.</i> 2015
44.	<i>T. panhai</i> (3)	KUHE:PH019	Phu Hin Rong Kla NP, Phitsa	-	AB830735	-	Nishikawa <i>et al.</i> 2013b
45.	<i>T. vietnamensis</i> (1)	IEBR 3243	Son Dong Dist, Bac Giang, Vietnam	HM770088	HM770088	-	Stuart <i>et al.</i> 2010
46.	<i>T. vietnamensis</i> (2)	IEBR 3244	Son Dong Dist, Bac Giang, Vietnam	HM770089	HM770089	-	Stuart <i>et al.</i> 2010
47.	<i>T. vietnamensis</i> (3)	KUHE:55171	Yen Tu, Bac Giang, Vietnam	-	AB769537	-	Nishikawa <i>et al.</i> 2013a
48.	<i>T. lizhenchangi</i>	KUHE:42316	Yizhang, Hunan, China	-	AB769532	-	Nishikawa <i>et al.</i> 2013a
49.	<i>T. liuyangensis</i>	CSUFT20100108	LiuYang, Hunan, China	-	KJ205598	-	Yang <i>et al.</i> 2014
50.	<i>T. broadoridgus</i>	CIB200085	Sangzhi, Hunan, China	-	KC147814	-	Nishikawa <i>et al.</i> 2013a
51.	<i>T. dabienicus</i> (1)	GNU110911002	Henan, China	-	JN934693	-	Unpublished
52.	<i>T. dabienicus</i> (2)	GNU110911001	Henan, China	-	JN934692	-	Unpublished
53.	<i>T. dabienicus</i> (3)	HNNUI0042024	Shangcheng, Henan, China	-	KC147812	-	Nishikawa <i>et al.</i> 2013a
54.	<i>T. dabienicus</i> (4)	HNNUI0042015	Shangcheng, Henan, China	-	KC147811	-	Nishikawa <i>et al.</i> 2013a
55.	<i>T. wenxianensis</i> (1)	-	-	EU880341	EU880341	EU880341	Zhang <i>et al.</i> 2008
56.	<i>T. wenxianensis</i> (2)	CIB20090527	Wenxian, Gansu, China	-	KC147813	-	Nishikawa <i>et al.</i> 2013a
57.	<i>T. wenxianensis</i> (3)	MVZ230352	Pingwu, Sichuan, China	DQ517855	DQ517855	-	Weisrock <i>et al.</i> 2006
58.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-001 **	Yuexi, Anhui Province, China	-	KY321388	-	This study
59.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-002 **	Yuexi, Anhui Province, China	-	KY321389	-	This study
60.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-003 **	Yuexi, Anhui Province, China	-	KY321390	-	This study
61.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-004 **	Yuexi, Anhui Province, China	-	KY321391	-	This study
62.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-005 †	Yuexi, Anhui Province, China	-	KY321392	-	This study
63.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-006 **	Yuexi, Anhui Province, China	-	KY321393	-	This study
64.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-007 **	Yuexi, Anhui Province, China	-	KY321394	KY321420	This study
65.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-008 †	Yuexi, Anhui Province, China	-	KY321395	-	This study
66.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-009 **	Yuexi, Anhui Province, China	-	KY321396	-	This study
67.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-010 **	Yuexi, Anhui Province, China	-	KY321397	-	This study
68.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-011 **	Yuexi, Anhui Province, China	-	KY321398	-	This study
69.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-012 **	Yuexi, Anhui Province, China	-	KY321399	KY321421	This study
70.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-013 †	Yuexi, Anhui Province, China	-	KY321400	-	This study
71.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-014 **	Yuexi, Anhui Province, China	-	KY321401	-	This study
72.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-015 **	Yuexi, Anhui Province, China	-	KY321402	-	This study
73.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-016 **	Yuexi, Anhui Province, China	-	KY321403	-	This study
74.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-017 **	Yuexi, Anhui Province, China	-	KY321404	-	This study
75.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-018 **	Yuexi, Anhui Province, China	-	KY321405	-	This study
76.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-019 **	Yuexi, Anhui Province, China	-	KY321406	KY321422	This study
77.	<i>T. anhuiensis</i> sp. nov.	AHU-16-EE-020 †	Yuexi, Anhui Province, China	-	KY321407	-	This study
78.	<i>T. anhuiensis</i> sp. nov.	AHU-13-EE-001 †	Yuexi, Anhui Province, China	-	KY321408	-	This study

(Continued Appendix)

ID	Species/ (No.) *	Voucher	Locality	GenBank accession NO.		Sequence Source
				ND1	ND2	
79.	<i>T. anhuiensis</i> sp. nov.	AHU-13-EE-002 †	Yuexi, Anhui Province, China	-	KY321409	This study
80.	<i>T. anhuiensis</i> sp. nov.	AHU-13-EE-003 **	Yuexi, Anhui Province, China	-	KY321410	This study
81.	<i>T. anhuiensis</i> sp. nov.	AHU-13-EE-004 **	Yuexi, Anhui Province, China	-	KY321411	This study
82.	<i>T. anhuiensis</i> sp. nov.	AHU-13-EE-005 **	Yuexi, Anhui Province, China	-	KY321412	This study
83.	<i>T. anhuiensis</i> sp. nov.	AHU-13-EE-006 **	Yuexi, Anhui Province, China	-	KY321413	This study †
84.	<i>T. anhuiensis</i> sp. nov.	AHU-13-EE-007 †	Yuexi, Anhui Province, China	-	KY321414	This study
85.	<i>T. anhuiensis</i> sp. nov.	AHU-13-EE-008 **	Yuexi, Anhui Province, China	-	KY321415	This study
86.	<i>T. anhuiensis</i> sp. nov.	AHU-13-EE-009 **	Yuexi, Anhui Province, China	-	KY321416	This study
87.	<i>T. anhuiensis</i> sp. nov.	AHU-15-EE-001 †	Yuexi, Anhui Province, China	-	KY321417	This study
88.	<i>T. anhuiensis</i> sp. nov.	AHU-13-EE-010 **	Yuexi, Anhui Province, China	-	KY321418	This study
89.	<i>T. anhuiensis</i> sp. nov.	AHU-13-EE-011 **	Yuexi, Anhui Province, China	-	KY321419	This study
90.	<i>Echinotriton andersoni</i>	MVZ232187	-	EU880314	EU880314	Zhang <i>et al.</i> 2008
91.	<i>E. chinhatensis</i>	TP26159	-	EU880315	EU880315	Zhang <i>et al.</i> 2008
92.	<i>Pleurodeles poireti</i>	MVZ235673	-	EU880329	EU880329	Zhang <i>et al.</i> 2008
93.	<i>P. waltl</i>	MVZ231894	-	EU880330	EU880330	Zhang <i>et al.</i> 2008

* indicates the number of different individuals from the same species in this study; † indicates the holotype of *T. anhuiensis* sp. nov.; ** indicates the male of *T. anhuiensis* sp. nov.; † indicates the female of *T. anhuiensis* sp. nov.